This work was supported by a research grant from Richardson-Vicks, Inc. and by Biomedical Research Support Grant No. 5-S07-RR-05413. The authors gratefully acknowledge Dr F. Joy Archer for her technical assistance.

REFERENCES

Al-Bazzaz, F. J. (1981) Am. Rev. Respir. Dis. 123: 295–298
Al-Bazzaz, F. J., Khan, A., Cheng, E. (1977) Clin. Res. 25: 414A
Barbieri, E. J., Bobyock, E., Chernick, W. S., McMichael, R. F. (1984) Br. J. Pharmacol. 82: 199–206
Barsigian, C., Barbieri, E. J. (1982) Agents Actions 12: 320–327
Berridge, M. J. (1975) Adv. Cyclic Nucleotide Res. 6: 1–98
Coles, S. J., Reid, L. (1981) Cell Tissue Res. 214: 107–118
Coles, S. J., Judge, J., Reid, L. (1982) Chest 81: 34S–36S
Davis, B., Marin, M. G., Yee, J. W., Nadel, J. A. (1979) Am. Rev. Respir. Dis. 120: 547–552
Goldberg, N. D., Haddox, M. K., Dunham, E., Lopez, C., Hadden, J. W. (1974) in: Clarkson, B., Baserga, R. (eds) Control of Proliferation in Animal Cells. Cold Spring Harbor Laboratory, pp 609–625
Heisler, S. (1974) Br. J. Pharmacol. 52: 387–392
Liedtke, C. M., Boat, T. F., Rudolph, S. A. (1982) Chest 82: 20S–21S
Marin, M. G., Zaremba, M. M. (1978) Fed. Proc. 37: 514
Phipps, R. J., Nadel, J. A., Davis, B. (1980) Am. Rev. Respir. Dis. 121: 359–365
Rubin, R. P. (1982) Calcium and Cellular Secretion. Plenum Press, New York
Shelhamer, J. H., Marom, Z., Kaliner, M. (1980) J. Clin. Invest. 66: 1400–1408
Wardell, J. R., Jr., Chakrin, L. W., Payne, B. J. (1970) Am. Rev. Respir. Dis. 101: 741–754

J. Pharm. Pharmacol. 1985, 37: 751–754
Communicated January 21, 1985

Induction of drug metabolizing enzymes in the liver of rats infested with *Fasciola hepatica*

P. GALTIER*, G. LARRIEU, P. LESCA†, *Laboratoire de Pharmacologie-Toxicologie INRA, 180 chemin de Tournefeuille, 31300 Toulouse, France and †Laboratoire de Pharmacologie et Toxicologie Fondamentales CNRS, 205 route de Narbonne, 31078 Toulouse Cédex, France*

Adult, male rats were infected with 20 metacercariae of *Fasciola hepatica* given orally, other rats were left untreated. Five weeks after infestation, some animals received phenobarbitone, 3-methylcholanthrene, β-naphthoflavone or Arochlor 1254, to induce liver drug metabolizing enzymes. Fascioliasis provoked decreases in aminopyrine *N*-demethylase, aniline hydroxylase, the mutagenic activity of cyclophosphamide and cytochrome P-450 concentration in untreated or phenobarbitone or Arochlor pretreated rats. In contrast, cytochrome b5, NADPH cytochrome c reductase, ethyoxycoumarin *O*-deethylase and the enzymatic activation of ethidium bromide were not affected by fascioliasis whatever pretreatment was given. Fascioliasis decreased liver drug metabolizing enzymes which were specifically induced by both phenobarbitone and Arochlor, this could be due to either the specific action of toxic excretions of flukes or to the particular localization of tissue damage within the liver lobule.

Hepatic microsomal cytochrome P-450 concentration and drug metabolizing enzymes fell 3 to 8 weeks after rats had been given orally 20 metacercariae of *Fasciola hepatica* (Galtier et al 1983).

The existence of several isozymes of rat liver cytochrome P-450 differing in substrate selectivity and immunological and physicochemical properties is now well established, the forms of cytochrome P-450 induced by phenobarbitone being shown not to be identical with those induced by 3-methylcholanthrene or β-naphthoflavone (Lu 1979).

* Correspondence.

We have investigated the effects on fluke infected rats of phenobarbitone, Arochlor 1254 (ARO), 3-methylcholanthrene or β-naphthoflavone with the aims of determining the inducibility of mixed function oxidases compared with the response in normal, uninfected rats and of characterizing the microsomal cytochrome P-450 species which are particularly inhibited five weeks after infection.

Materials and methods

Phenobarbitone, 3-methylcholanthrene (Serva, Heidelberg, GFR), Arochlor 1254 (Alltech, Eke, Belgium), β-naphthoflavone (Ega-Chemie, Steinheim, GFR), ethoxycoumarin (Boehringer-Manneim, Meylan, France), ethidium bromide and cyclophosphamide (Sigma, St Louis, USA) were used as received.

Treatment of animals. Male, Sprague Dawley rats, about 150 g, were randomly distributed into control or infected groups of 8 rats housed in cages of 4 animals. Food (UAR alimentation, Villemoisson, France) and drinking water were freely available. Each rat to be infected received by gastric tube 20 metacercariae of *Fasciola hepatica* suspended in a 1% polysorbate aqueous solution. The development of the condition was observed clinically and by means of biochemical studies of blood samples. Parallel studies were made on uninfected controls, which received no treatment, since

it had been shown that treatment by vehicle only did not modify monooxygenase activities.

Five weeks after infection, phenobarbitone (80 mg kg^{-1}) dissolved in 0·9% NaCl (w/v) was administered intraperitoneally once a day for 3 days or Arochlor (200 mg kg^{-1}), 3-methylcholanthrene or β-naphthoflavone (each 80 mg kg^{-1}), were dissolved in corn oil, and injected as a single dose. Phenobarbitone-, Arochlor-, 3-methylcholanthrene- or β-naphthoflavone-treated rats were killed at 24, 120 and 48 h, respectively, after the last injection and the abdomen opened and the blood drawn by puncture through the abdominal aorta. The liver was removed immediately, freed of extra-hepatic tissue, blotted free of excess moisture and weighed (0–4 °C).

9000*g* supernatant and hepatic microsomes were prepared by differential centrifugation and stored as previously described (Galtier et al 1983).

Assays. The activity of plasma glutamic oxaloacetic transaminase (GOT) was estimated at 37 °C by the method of Reitman & Frankel (1957) by means of Biochemical Test Combination (Boehringer Mannheim, ref 125881). The microsomal protein concentration was determined according to the method of Lowry et al (1951) with bovine serum albumin as the standard. Cytochromes P-450 and b5 were analysed according to Omura & Sato (1964). The activity of NADPH-cytochrome c reductase was determined by measuring spectrophotometrically the rate of reduction of cytochrome c (Mazel 1971). Aniline hydroxylase and aminopyrine demethylase activities were measured according to Mazel (1971). The formation of formaldehyde from demethylated aminopyrine was estimated by the method of Nash modified by Cochin & Axelrod (1959). Ethoxycoumarin *O*-deethylase was determined using the method of Aitio (1978). The mutagenic effects of ethidium bromide or cyclophosphamide allowing the specific detection of 3-methylcholanthrene- and phenobarbitone-induced forms of cytochrome P-450 were analysed by the dual assay of Lesca et al (1984).

Statistics. Direct comparisons to a single control were conducted by the unpaired Student's *t*-test and multiple comparisons to a single control were made with Dunnett's test (1964).

Results

At autopsy, lesions characteristic of long-standing fascioliasis were present in the livers of all infected rats; the hepatic parenchyma appeared to be permeated by dark haemorrhagic foci as already described for a 5 week-infection (Galtier et al 1983). Liver injury was assessed by determining elevation of plasma activity of GOT. Of the four enzyme inducers, only Arochlor caused a 1·5 fold elevation in GOT activity (Table 1), whereas fascioliasis provoked increases in this activity in all infected groups except in those pretreated with Arochlor. Phenobarbitone, Arochlor, 3-methylcholanthrene and β-napththoflavone caused a significant increase in liver to body weight ratio (4·57–5·80%) compared with the uninfected controls (3·99 ± 0·12%); fascioliasis did not modify these values. There was no change in liver microsomal protein concentrations whatever the pretreatment and the infective state (Table 1).

Significant increases in the concentration of microsomal cytochrome P-450 were observed in rats pretreated with phenobarbitone (155%) Arochlor (202%), 3-methylcholanthrene (42%) or β-naphthoflavone (37%) compared with untreated controls. With microsomes from 3-methylcholanthrene and β-naphthoflavone pretreated rats, the maximal absorption of reduced cytochrome P-450-CO complex (by difference

Table 1. Effects of inducers in control or 5 week-infected rats on plasma and liver parameters (mean ± s.e.m. of 8 rats).

Treatment	Pathology	Plasma GOT (unit ml^{-1})	Microsomal proteins (mg g^{-1})	Liver Cytochrome P-450 (nmol mg^{-1}micr. proteins)	Liver Cytochrome b5 (nmol mg^{-1})	NADPH cytochrome c reductase (nmol min^{-1} mg^{-1})
None (control)	Uninfected	40·8 ± 3·0	25·0 ± 1·3	0·549 ± 0·023	0·307 ± 0·018	53·1 ± 4·4
	Infected	69·9 ± 4·1†	23·7 ± 1·4	0·460 ± 0·022†	0·328 ± 0·023	50·6 ± 2·9
Phenobarbitone	Uninfected	42·9 ± 2·5	28·1 ± 1·3	1·405 ± 0·055*	0·272 ± 0·030	83·0 ± 3·7*
	Infected	72·9 ± 11·5†	27·8 ± 3·0	1·051 ± 0·067†	0·302 ± 0·023	77·4 ± 4·3
Arochlor 1254	Uninfected	64·5 ± 10·5*	29·2 ± 1·7	1·656 ± 0·048*	0·285 ± 0·022	71·6 ± 7·9*
	Infected	74·8 ± 7·5	28·1 ± 3·8	1·173 ± 0·113†	0·312 ± 0·029	74·3 ± 4·0
3-Methylcholanthrene	Uninfected	51·9 ± 4·9	27·1 ± 0·8	0·780 ± 0·031*	0·348 ± 0·022	60·3 ± 6·0
	Infected	80·5 ± 7·8†	25·6 ± 2·3	0·697 ± 0·065	0·364 ± 0·033	55·7 ± 3·5
β-Naphthoflavone	Uninfected	48·0 ± 3·6	26·4 ± 1·1	0·755 ± 0·052*	0·332 ± 0·018	53·7 ± 2·8
	Infected	86·2 ± 10·7†	23·4 ± 1 2	0·739 ± 0·070	0·380 ± 0·029	54·3 ± 6·7

The results have been statistically analysed, * indicates a significant difference ($P < 0·05$) between control and pretreated rats, and † indicates a significant difference ($P < 0·05$) between uninfected and corresponding infected rats.

Table 2. Effect of inducers in controls or 5 week-infected rats on hepatic drug metabolizing enzymes (mean ± s.e.m. of 8 rats).

Treatment	Pathology	Aminopyrine *N*-demethylase (nmol $min^{-1} mg^{-1}$)	Aniline hydroxylase (nmol $min^{-1} mg^{-1}$)	Ethoxycoumarin *O*-deethylase (nmol $min^{-1} mg^{-1}$)	Ethidium bromide mutagenic activation (His† revertants per plate)	Cyclophosphamide mutagenic activation (His† revertants per plate)
None (control)	Uninfected	1·36 ± 0·09	0·83 ± 0·06	0·13 ± 0·04	80 ± 2	162 ± 13
	Infected	0·57 ± 0·07†	0·49 ± 0·12†	0·14 ± 0·05	70 ± 2	164 ± 24
Phenobarbitone	Uninfected	2·29 ± 0·10*	1·15 ± 0·08*	0·39 ± 0·09*	—	1076 ± 196*
	Infected	1·24 ± 0·17†	0·88 ± 0·08†	0·30 ± 0·04	—	649 ± 59†
Archlor 1254	Uninfected	1·53 ± 0·08*	1·14 ± 0·07*	0·31 ± 0·02*	1108 ± 195*	—
	Infected	0·75 ± 0·11†	0·64 ± 0·06†	0·45 ± 0·17	1267 ± 288	—
3-Methylcholanthrene	Uninfected	1·08 ± 0·11	0·82 ± 0·06	0·34 ± 0·07*	1414 ± 529*	—
	Infected	0·33 ± 0·06†	0·54 ± 0·09†	0·43 ± 0·08	1328 ± 338	—
β-Naphthoflavone	Uninfected	1·18 ± 0·13	0·87 ± 0·04	0·33 ± 0·05*	225 ± 46*	—
	Infected	0·47 ± 0·15†	0·47 ± 0·16†	0·44 ± 0·11	476 ± 302	—

For legends see Table 1.

spectra measurements) was observed at 448 nm. Fascioliasis significantly decreased the cytochrome P-450 concentrations in microsomes from untreated rats (16%) and from rats given phenobarbitone (25%) or Arochlor (29%); the injection did not affect the enhanced cytochrome P-450 level in liver microsomes from rats pretreated with 3-methylcholanthrene or β-naphthoflavone. Neither inducer pretreatment nor infection affected the concentration of cytochrome b5. However, NADPH cytochrome c reductase activities were increased in rats pretreated with phenobarbitone (56%) or Arochlor (35%), but these increases were not altered by fascioliasis in the corresponding infected groups.

The activities of both microsomal aminopyrine *N*-demethylase and aniline hydroxylase were increased in rats given phenobarbitone (68 and 39%, respectively) or Arochlor (13 and 37%) compared with untreated controls or other uninfected groups (Table 2); in all infected groups, the activities of these two enzymes decreased by 46–68% and 23–46%, respectively. Ethoxycoumarin *O*-deethylase activity was increased 2·4 to 3 fold in pretreated rats, whichever pretreatment was used, and these increases were not reduced in corresponding infected groups.

Table 2 includes the results of enzymatic activation of ethidium bromide and cyclophosphamide to their mutagenic metabolites. As reported by Lesca et al (1984), the activation of ethidium bromide was induced 3- to 18-fold by β-naphthoflavone, Arochlor or 3-methylcholanthrene, whereas the activation of cyclophosphamide was induced 6·6 fold in 9000*g* supernatant from the livers of phenobarbitone-treated animals. There was a significant decrease (40%) in activation to mutagenic metabolites caused by fascioliasis in phenobarbitone-pretreated rats compared with results from corresponding uninfected animals.

Discussion

We have found that in untreated rats, fascioliasis provokes an increase in plasma GOT activities and a significant decrease in microsomal cytochrome P-450 concentrations and in drug metabolizing enzymes such as aminopyrine *N*-demethylase or aniline hydroxylase. It did not affect either NADPH cytochrome c reductase or ethoxycoumarin *O*-deethylase microsomal activities in any group of rats. NADPH cytochrome c reductase has been shown to be unchanged in liver diseases (experimental ischaemia) in which significant decreases in cytochrome P-450, aminopyrine *N*-demethylase and aniline hydroxylase activities were observed (Ferrero et al 1978).

As expected, phenobarbitone pretreatment caused a significant induction in microsomal cytochrome P-450, aminopyrine *N*-demethylase and aniline hydroxylase concentrations. NADPH cytochrome c reductase and ethoxycoumarin *O*-deethylase activities were also increased (Alvares 1977; Miranda & Chhabra 1980). The mutagenic activation of cyclophosphamide by liver microsomes was also increased after treatment of rats with phenobarbitone as found by Hales & Jain (1980). Because of similarities in the physical and catalytic properties of the 3-methylcholanthrene-inducible cytochrome P-448 and the β-naphthoflavone-inducible cytochrome P-446 (Lau et al 1982), increases in microsomal cytochrome P-448 concentrations, ethoxycoumarin *O*-deethylase and enzymatic activation of ethidium bromide to mutagenetic derivatives were similar in the treated rats. However, as previously shown in adult male, Sprague Dawley rats, 3-methylcholanthrene pretreatment, in contrast to phenobarbitone, did not induce either aminopyrine *N*-demethylase or aniline hydroxylase (Iba & Sikka 1983). Arochlor 1254, a mixture of polychlorobiphenyls, induced both the phenobarbitone and 3-methylcholanthrene type effects in the liver of male rats (Alvares 1977). Like phenobarbitone, Arochlor pretreatment caused a significant increase in aminopyrine *N*-demethylase, aniline hydroxylase and NADPH cytochrome c reductase. It also enhanced the enzymatic activation of ethidium bromide to reactive mutagenetic species as demonstrated by Lecointe et al (1981), as also reported for β-naphthoflavone and 3-methylcholanthrene (Lesca et al 1984).

The effect of fascioliasis on the induction of microsomal drug metabolizing enzymes by various chemicals

administered to rats consisted of decreasing the microsomal cytochrome P-450 specifically induced by both phenobarbitone and Arochlor. Consequently the activities of aminopyrine n-demethylase and aniline hydroxylase and the enzymatic activation of cyclophosphamide were inhibited while the infection did not affect ethoxycoumarin *O*-deethylase or the activation of ethidium bromide. From these results it may be inferred that the modifications caused by *Fasciola hepatica* on the function of the hepatic cells do not concern the Ah receptor-dependent induction process which is specific for many polyaromatic chemicals such as 3-methylcholanthrene, β-naphthoflavone, some biphenyl derivatives and dioxin. In contrast, the pleiotypic induction of structural (smooth endoplasmic reticulum) as well as enzymatic proteins by phenobarbitone appears to be much more sensitive to fascioliasis. These various effects underline the fundamental difference probably existing between the mechanisms of the induction process triggered by either phenobarbitone-like or methylcholanthrene chemicals.

The reason for the specific alteration of particular isozymes of cytochrome P-450 could be related either to the specific action of toxic excretions of the flukes in the course of their histophagous migration through the liver parenchyma (Jubb & Kennedy 1970) or to the particular localization of these enzymes surrounding the nuclei in the hepatic centrilobular zones as demonstrated by means of immunofluorescence (Ohnishi et al 1982).

The authors would like to thank Professor M. Franc (Ecole Nationale Vétérinaire de Toulouse) for providing metacercariae of *Fasciola hepatica*, Mrs Eeckhoutte and Mr Tufenkji for skilful assistance in statistical and biochemical analysis.

REFERENCES

Aitio, A. (1978) Anal. Biochem. 85: 488–491

Alvares, A. P. (1977) in: Ullrich, V., Roots, I., Hildebrandt, A., Estabrook, R. W., Conney, A. H. (eds) Microsomes and Drug oxidations. Pergamon, Oxford, pp 476–483

Cochin, J., Axelrod, J. (1959) J. Pharmacol. Exp. Ther. 125: 105–115

Dunnett, C. W. (1964) Biometrics 6: 482–489

Ferrero, M. E., Oris, R., Bernelli-Zazzera, A. (1978) Exp. Mol. Pathol. 28: 256–266

Galtier, P., Battaglia, A., Moré, J., Franc, M. (1983) J. Pharm. Pharmacol. 35: 729–733

Hales, B. F., Jain, R. (1980) Biochem. Pharmacol. 29: 2031–2037

Iba, M. M., Sikka, H. C. (1983) Ibid. 32: 901–909

Jubb, K. V. F., Kennedy, P. C. (1970) Pathology of Domestic Animals, vol. 2, Academic Press, New York, pp 241–246

Lau, P. P., Pickett, C. B., Lu, A. Y. H., Strobel, H. W. (1982) Arch. Biochem. Biophys. 218: 472–477

Lecointe, P., Bichet, N., Fraire, C., Paoletti, C. (1981) Biochem. Pharmacol. 30: 601–609

Lesca, P., Fournier, A., Lecointe, P., Cresteil, T. (1984) Mutation Res. 129: 299–310

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275

Lu, A. Y. H. (1979) Drug Metab. Rev. 10: 187–208

Mazel, P. (1971) in: La Du, B. N., Mandel, H. G., Way, E. L. (eds) Fundamentals of Drug Metabolism and Drug Disposition. Williams and Wilkins Co., Baltimore, pp 527–545

Miranda, C. L., Chhabra, R. S. (1980) Biochem. Pharmacol. 29: 1161–1165

Ohnishi, K., Mishima, A., Okuda, K. (1982) Hepatology 2: 849–855

Omura, T., Sato, R. (1964) J. Biol. Chem. 239: 2370–2378

Reitman, S., Frankel, S. (1957) Am. J. Clin. Pathol. 28:56–64